Role of Noncoding RNA in Vascular Remodelling

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Purpose of review: Non-coding RNA (ncRNAs), such as microRNAs (miRNAs) and long non-coding RNAs (LncRNAs), are becoming fundamentally important in the pathophysiology relating to injury-induced vascular remodelling. We highlight recent studies that demonstrate the involvement of ncRNAs in vein graft disease, in in-stent restenosis (ISR) and in pulmonary arterial hypertension (PAH), with a particular focus on endothelial cell (EC) and vascular smooth muscle cell (VSMC) function. We also briefly discuss the emerging role of exosomal-derived ncRNAs and how this mechanism impacts on vascular function.

Recent findings: NcRNAs have been described as novel regulators in the pathophysiology of vascular injury, inflammation and vessel wall remodelling. In particular, several studies have demonstrated that manipulation of miRNAs can reduce the burden of pathological vascular remodelling. Such studies have also shown that exosomal miRNA-mediated, cell-to-cell communication between ECs and VSMCs is critical in the disease process. In addition to miRNAs, lncRNAs are emerging as regulators of vascular function in health and disease. Although lncRNAs are complex in both their sheer numbers and mechanisms of action, identifying their contribution to vascular pathology is essential.

Summary: Given the important roles of ncRNAs in vascular injury and remodelling together will their capacity for cell-to-cell communication, manipulating ncRNA might provide novel therapeutic interventions.

Keywords: Non-coding RNA, Vascular remodelling, in-stent restenosis, vein graft, pulmonary arterial hypertension

Introduction

Vascular remodelling after acute injury is a multifactorial pathological process that contributes to cardiovascular disease, including ISR, PAH, atherosclerosis, and others [1, 2]. The remodelling process involves structural changes of the vessel as a consequence of altered cell proliferation/growth, cell apoptosis/death, cell migration, and modulation of the production and degradation of extracellular matrix (ECM) [3, 4]. Both endothelial and smooth muscle cells are key mediators of the pathological response to acute vascular injury, driving the progressive remodelling process that characterizes neointimal formation [5, 6]. In addition, inflammatory activation of the vessel wall also contributes to vascular remodelling [7]. Endothelial injury, such as the endothelial denudation that arises after implantation of stents into coronary vessels, is an important early contributor to the initiation of vascular inflammation [8]. Following injury, growth factors and cytokines released from ECs and inflammatory cells promote smooth muscle cell proliferation, migration and ECM protein deposition [9, 10]. Although multiple studies over the last two decades have focused on delineating the molecular mechanisms that drive the remodelling response to vascular injury [11-13], with the exemption of drug-eluting stents, there is currently a paucity in effective therapeutics, necessitating further research and innovative thinking around molecular mechanisms that might govern pathological responses

According to recent transcriptome analyses, protein-coding genes actually account for only 1.5%-2.0% of the human transcriptome. The transcriptional output of the larger portion of the transcriptome (i.e. ~ 98%) consists of non-coding RNA species, including miRNAs (< 200nt) and LncRNAs >200nt [14]. MiRNAs have been intensively studied for the last decade [15, 16], However, lncRNA structure and function is an emerging field in the vascular arena, with the publication of several studies recently that suggest an important contribution to pathological vascular remodelling processes.

MiRNA biogenesis and function

MiRNAs are a novel class of endogenous, small/short non-coding transcripts of 16 to 29 nucleotides in length RNA that negatively regulate gene expression via degradation or translational inhibition of their target mRNAs. The biogenesis of miRNA is well reviewed in several highly cited publications [17-19]. MiRNAs are regarded as essential regulators in physiology and pathophysiology of the cardiovascular system [20, 21]. They are becoming an intriguing target for therapeutic intervention for a number of reasons. First, a single miRNA can regulate several hundred target genes (including targets in the same or complimentary cellular pathways) making miRNAs potentially very powerful disease modifiers [22]. Second, several lines of evidence demonstrate that miRNAs are essential regulators of VSMC development, differentiation, and contractile function offering a rich repertoire of disease-modifying therapeutics [23]. Finally, RNA therapeutics benefit from a great deal of prior translational research (e.g. SiRNA and exon skipping technology) that suggest RNA therapeutics might be attractive, albeit with specific challenges such as delivery, off target effects etc. [24].

The role of miRNAs in pathological vascular remodelling

Neointimal formation is a hallmark of many vascular diseases. VSMCs are one of the major cellular components of the vessel wall and are implicitly involved in neointimal formation [25]. For example (and aligned to our own research) in vein graft disease, VSMC proliferation and migration contributes to the increased vein graft medial thickening, neointimal formation and superimposed atherosclerosis [26]. Similarly, the proliferation of VSMC in response to vascular injury following angioplasty and stent implantation leads to neointimal formation and restenosis [27]. Further, a key feature of PAH is pulmonary vascular remodelling and development of occlusive and plexiform intimal lesions in the small, distal pulmonary vessels [28]. In this section, we discuss recent findings on the regulation of pathological vascular remodelling by miRNAs.

MiR-21 first identified to be significantly increased in rat carotid arteries following balloon injury [29]. In VSMCs, knockdown of miR-21 inhibited cell proliferation, decreased migration and induced apoptosis by targeting important regulators of cellular function, including PTEN and Bcl-2 [29], PDCD4 and activator protein 1 [30], tropomyosin 1 [31], while overexpression of miR-21 induced human aortic SMC proliferation and reduced VSMC differentiation by targeting specificity protein-1 (SP1) [32]. Landmark studies also showed that miR-21 is involved in the regulation of TGF-β and BMP signalling, activation of which led to increased expression of miR-21 via post-transcriptional mechanisms [33, 34]. These signalling pathways are central mediators of the vascular remodelling process following acute injury, regulating VSMC proliferation, migration, and phenotype [35]. Similar to the Ji *et al* study described above, upregulation of miR-21 has been observed across many *in vivo* models of vascular injury, including mouse and pig models of vein graft failure, and mouse models of ISR [29] [36] [37**] [38**]. These studies also underlined the therapeutic potential of targeting this broadly-expressed miRNA, showing that pharmacological inhibition of miR-21 could decrease neointimal formation in injured rat carotid arteries following angioplasty, while anti-miR-21 coated stents effectively reduced ISR by inhibiting VSMC proliferation following implantation [29] [38**]. Consistent with these findings, genetic ablation of miR-21 significantly reduced neointimal formation in a mouse vein graft failure model[36] and blocked neointimal formation in a mouse model of ISR by reducing VSMC proliferation and migration, macrophage polarization, and inflammatory activation[37**]. Mechanistic studies have identified several target genes of miR-21, suggesting complex mechanism of action dictated by both expression across a range of cell types and targeting transcriptome within those cells in a dynamic, pathologically regulated manner [36-38**]. In addition, the expression of miR-21 in plasma samples from patients with ISR following implantation of drug-eluting stents were significantly higher than in non-ISR patients and healthy controls, which showed that miR-21 could act as a potential biomarker for the development of ISR [39]. In PAH, manipulation of miR-21 levels has also been shown to affect the vascular remodelling that leads to the formation of obstructive lesions in the pulmonary vasculature. Sequestration of miR-21 by antimiR-21 treatment diminished chronic-hypoxia-induced pulmonary hypertension (PH) and attenuated pulmonary vascular remodelling [40]. However, miR-21 knockout mice displayed exaggerated manifestations of PH in SU5416 and chronic hypoxia models [41*, 42]. This data, while conflicting, highlights the complex role that miR-21 might play in vascular remodelling in the distal vasculature in the lung. Further studies are now required to identify the mechanisms underlying these divergent phenotypes, potentially by exposing miR-21 conditional knockout animals to different experimental PAH induction protocols, or by utilising novel strategies to inhibit miR-21 *in situ*. The role of miR-21 in PAH has been extensively reviewed elsewhere [43]. In a translational context, manipulation of miR-21 in different pre-clinical disease models has shown positive therapeutic effects, although it would be important to develop strategies for localised targeting of the miR-21 inhibitors to the vessel wall, such as in the case of eluting stents. Interestingly, a chemically modified oligonucleotide antimiR-21 manufactured by Regulus Therapeutics (San Diego, CA92121, United States) has entered phase 1 clinical trials for the treatment of Alport Syndrome, a disease of the glomerular basement membrane, where miR-21 inhibition is aimed at targeting abnormal renal function [44].

In addition to miR-21, several other miRNAs have been shown to play critical roles in the pathological response to acute vascular injury. MiRNAs have been shown to mediate the effects of many known regulators of vascular disease, such as PDGF, TGF-β/BMP, and the interleukin family of inflammatory cytokines. For example, miR-146b-5p is necessary for PDGF-induced VSMC phenotype transition [45]. MiRNAs that control vascular functions by affecting TGF-β family signalling have been reviewed elsewhere [46]. MiRNAs also play a central role in the modulation of vascular cell behaviour and function during disease pathogenesis, such as Let-7a and miR-221in vein graft disease [47] [48], the miR-143/145 cluster in ISR and PAH [49] [39] [50**] and miR-30c [51], miR-322 [52], miR-130a [53] in PAH. An overview of confirmed target genes for these miRNAs are showed in Table 1.

In summary, many miRNAs are now known to be associated with the development of vascular remodelling processes *in vivo* and *in vitro*, while manipulation of several miRNAs have shown therapeutic efficacy in animal models of vascular disease. However, many facets of miR biology remain to be fully elucidated, including their precise mechanisms of action, cell selectivity in effect and the factors underlying their diverse effects. Moreover, as previously stated, there is currently a lack of effective pharmacological strategies for the direct targeting of miRNA in disease pathogenesis. Thus, much works remains to be done to fully understand, target and therapeutically manipulate the biological roles of miRNAs in vascular disease.

LncRNA transcription and function

LncRNAs, in common with mRNAs, are generally transcribed by RNA Polymerase II, and further spliced, 5’-capped (m7G), and 3’-polyadenylated [54]. The transcriptional regulation of lncRNA is well reviewed elsewhere [55]. LncRNA are, in general, expressed at relatively low levels and are far less well conserved than miRNA [56-58]. LncRNA can be classified into different subtypes based on their genomic location: sense lncRNAs, antisense lncRNAs, bidirectional lncRNAs, intronic lncRNAs and intergenic lncRNAs [59]. LncRNAs have diverse molecular mechanisms of regulating gene expression through interactions with DNA, RNA, or protein, by acting as decoys, scaffolds, guides or signalling molecules (Figure 1). In addition, lncRNAs can regulate gene expression post-transcriptionally, for example by modulating splicing, mRNA translation and mRNA degradation, or by acting as miRNA sponges. There are several comprehensive reviews summarized the functions and mechanisms of lncRNA [55, 60-63].

Role of lncRNAs in vascular remodelling

Accumulating early evidence suggests that lncRNAs may play an important role in vascular pathophysiology. Early studies identified antisense noncoding RNA in the INK4 locus (ANRIL), a lncRNA expressed in VSMC, which was able to regulate CDKN2A/B expression in aortic VSMC and attenuate proliferation in atherosclerosis [64-66] as well as modulate inflammatory responses in coronary artery disease [67].

Lnc-Ang362, which hosts the miR-221 and miR-222 genes, was shown to be regulated by angiotensin II in VSMC, and was in turn able to modulate VSMC proliferation, vascular remodelling and neointimal hyperplasia in concert with miR-221 and miR-222 [68-70]. These studies showed that lncRNA were involved in the control of cell proliferation, and could be regulated alongside proximal miRNAs in VSMCs. RNA-Seq analysis of human coronary artery SMC revealed a novel lncRNA, Smooth muscle and Endothelial cell-enriched migration/differentiation-associated long NonCoding RNA (SENCR), which is expressed in both VSMCs and ECs In VSMCs, knockdown of SENCR decreased the expression of myocardin and numerous smooth muscle contractile genes and increased a number of pro-migratory genes (namely Midkine (MDK) and Pleiotrophin (PTN)). Inhibition of these two genes reversed the increased VSMC migration mediated by SENCR silencing, which suggested that the expression of these genes was partially regulated by SENCR in these cells. However, the functions of SENCR in endothelial cells were not reported in this study [71*]. Our group further revealed that SENCR induced the proliferation, migration and angiogenesis of Human Umbilical Vein Endothelial Cells (HUVECs). In patient samples, SENCR expression was altered in vascular tissues and cells derived from patients with critical limb ischemia and premature coronary artery disease compared with healthy controls [72]. These two studies revealed that SENCR could modulate the behaviour of both SMC and EC, and exhibited altered expression patterns in vascular tissues and cells from vascular disease patients. However, the exact molecular targets and mechanisms of

SENCR in pathological vascular remodelling remain to be determined.

Similar to SENCR, LincRNA-p21 is expressed in both VSMC and EC. In human VSMCs, lincRNA-p21 repressed cell proliferation and induce apoptosis *in vitro*. LincRNA-p21 also inhibited neointimal formation in carotid arteries and repressed proliferation and induced apoptosis in carotid artery injury model *in vivo*. The authors further confirmed the expression level of lincRNA-p21 was decreased in patients with coronary heart disease [73**]. In mouse EC, lincRNA-p21 induced cell apoptosis and cell cycle progression. In addition, lincRNA-p21 inhibited cell proliferation through binding miR-130b [74]. Unlike many lncRNAs, LincRNA-p21 contains a very highly conserved 780 nt length 5' region in mammals, which provided the possibility of using an *in vivo* animal model to study the role of this lncRNA [75]. These data identified lincRNA-p21 is a key regulator of cell proliferation and apoptosis in vascular cells, which are primary contributors to vascular remodelling. Consistent with the human *in vitro* data, knockdown of lincRNA-p21 resulted in dramatic neointimal hyperplasia in a mouse model of carotid artery injury, which indicated that lincRNA-p21 could serve as therapeutic target to treat vascular remodelling diseases [73**].

Using RNA-Seq, we recently defined a novel smooth muscle specific lncRNA SMILR, which could be induced by IL-1α and PDGF in human saphenous vein smooth muscle cells (HSVSMCs). Knockdown of SMILR markedly reduced HSVSMC proliferation, likely through regulation of the HAS2 gene, located proximal to the genetic locus for SMILR. In human samples, SMILR expression increased in unstable atherosclerotic plaques and plasma from patients with high plasma C-reactive protein [76**]. These data suggested that inhibiting SMILR expression could be a potential target for treating vascular diseases characterised by VSMC proliferation.

In the context of EC, studies have shown that the lncRNA Metastasis associated lung adenocarcinoma transcript 1 (MALAT1) could regulate retinal vessel remodelling by affecting retinal EC proliferation, migration and tube formation, as genetic ablation of MALAT1 inhibited the proliferation of EC and reduced neonatal retina vascularization *in vivo* [77, 78**]. In human aortic EC, knockdown of the lncRNA H19 reduced cell proliferation and activated p21/CDKN1A, increasing apoptosis, decreasing angiogenesis, and diminishing the number of cells in G0/G1 and S phases in cell cycle [79]. These two lncRNAs that are highly expressed in EC showed multifunctional features both *in vivo* and *in vitro*. EC lncRNAs have been also reviewed elsewhere [80-82]. An overview of target genes/miRNAs of these lncRNAs is given in Table 1.

In summary, there are few lncRNAs that have been studied in depth in the context of vascular disease, with most studies simply reporting the dysregulation of vascular cell function after manipulation of the lncRNAs. The direct targets and mechanisms of many lncRNA remain largely unknown, so urgent attention is needed in this context, particular relating to their mechanism of action. Unfortunately, the low conservation of many lncRNAs restricts the availability of *in vivo* disease models, which is a significant impediment to translation of lncRNA therapeutics to the clinical arena. Therefore, much more work is required to fully explore the role of lncRNAs in the pathophysiological processes driving vascular disease, sometimes using innovative models with human cells and tissues.

Vascular cell-to-cell communication through miRNAs

Cell-to-cell communications can be mediated by extracellular vesicles, exosomes, apoptotic bodies, microparticles, and tunnelling nanotubes (TNTs) [83, 84]. Exosomes are 40 to 100nm in size and represent a specific subtype of secreted membrane vesicles formed through the fusion of multivesicular endosomes with the plasma membrane [85]. Exosomes are released by many types of cell, and have been identified in most body fluids, including plasma, urine, saliva, cerebrospinal, amniotic and synovial fluids. Exosomes are now known to carry a wide array of molecules including: proteins, DNAs, mRNAs, miRNAs and lncRNAs, depending on a variety of factors including the cell type from which the exosome originates, the state of health of the host, and extracellular stimuli [86]. Exosomes can be taken up by neighbouring or distant cells and can be functional in the recipient cells by fusion with the plasma membrane, via receptor-mediated uptake or by internalisation via endocytosis or macropinocytosis [87]. Since the discovery of miRNA in exosomes, a number of studies have focused on the identification and function of exosomal miRNAs in cancer [88] and cardiovascular diseases [89-93]. Interestingly, cancer cell derived exosomes contain key components of miRNA biogenesis including the proteins RNA-induced Silencing Complex (RISC), Argonaute-2 (AGO2), Dicer, and

TAR RNA-binding protein (TRBP), which displayed cell-independent capacity to process precursor miRNAs into mature miRNAs [94]. Here we focus on the role of exosomal miRNAs in vascular cells.

An early study showing the importance of exosomal miRNAs in vascular cells evaluated exosomal miR-143/145 in atherosclerosis. This study showed that miR-143/145 can affect vascular cell function following exosomal transport, via cell-to-cell communication. Kruppel like factor 2 (KLF2) or shear-stimulated HUVECs secreted miR-143/145 enriched exosomes, which could be taken up by VSMCs to derepress the targets genes of miR-143/145 (such as EST domain-containing protein Elk-1 (ELK1), Krupple like factor 4 (KLF4), Matrix metallopeptidase 3 (MMP3), Slingshot protein phosphtase 2 (SSH2)) and induce an atheroprotective SMC phenotype both *in vitro* and *in vivo* [95]. In the pulmonary vasculature, we recently found that exosomal miR-143 derived from pulmonary artery smooth muscle cells (PASMCs) can be transferred to pulmonary artery endothelial cells (PAECs) exerting pro-migratory and pro-angiogenic effects [50**]. Apart from exosome-mediated transfer, TGF-β triggered miR-143/145 transfer to ECs from VSMCs via TNTs. Over-expression of miR-143/145 in ECs had anti-angiogenic effects and reduced EC proliferation by directly targeting hexokinase 2 (HKII) and Integrin beta-8 (ITGβ8) separately [96**]. These data demonstrated the vital role of miR-143/145 in vascular cell proliferation, migration, and angiogenesis. Other EC secreted miRNAs including miR-126 that could be taken by VSMCs via Ago2-facilitated miRNA transmission [97], miR-26a [98*] and miR-214 [99]. The cell-to-cell communication between vascular cells by miRNAs is summarised in Figure 2. This is a relatively new field and there is much to discover about miRNA regulation and function through secretion and cell: cell communication.

Exosomal lncRNAs have been found in several cancer cells and patient plasma. These include lncRNA HOX transcript antisense RNA (HOTAIR) [100], H19 [101], Long intergenic Non-coding RNA, Regulator Of Reprogramming (lncRNA ROR) [102], Linc-very-low-density lipoprotein receptor (Linc-VLDLR) [103], lncRNA transcript UC.339 (lncRNA TUC339) [104], lncRNA-p21 [105] and other lncRNAs [106]. However, there is no report about exosomal lncRNAs secreted by the vascular cells. Recently, our group demonstrated that smooth muscle enriched lncRNA Smooth muscle enriched long noncoding RNA (SMILR) was secreted into the cell culture media but was located in a non-exosomal or microparticle fraction [76**]. More studies should be performed to investigate the secretory pathways utilised by this lncRNA, the function of the secreted lncRNA molecules and the compartment or fraction that the lncRNA is located within. More generally, it would be important to identify whether cell-cell communication mediated by exosomal or microparticle lncRNAs plays a role in regulating the behaviour of vascular cells during vascular remodelling processes.

Therapeutic prospective of ncRNAs

The translation of ncRNAs from bench to bedside has received considerable attention in recent years. In general, the therapeutic approaches involve the inhibition or overexpression of ncRNAs. Several approaches to manipulate ncRNAs are currently in use pre-clinically, including miRNA antagonists, miRNA mimics, antisense oligonucleotide (ASO), and small interfering RNA (siRNA). The first anti-miRNA (Miravirsen) to enter clinical trials is a locked nucleic acid-modified DNA phosphorothioate antisense oligonucleotide targeting miR-122, which sequesters mature miR-122 to treat patients with hepatitis C. Miravirsen is currently in phase 2 clinical trials [107, 108]. MiR-RX34 (MRX34), a double-stranded miR-34 RNA mimic encapsulated in a liposomal nanoparticle formulation, is the first miRNA mimic to enter phase I clinical trials [109]. Several antimiRNA/mimic therapeutics targeting miRNAs are currently under development, including Let-7, miR-21, miR-208, miR-195, miR-221, miR-29, miR-155, miR-10b, and miR-103/107. Four of these miRNA-based therapeutic drugs have entered into phase 1 clinical trials [110-114*]. Despite intensive studies of miRNAs in vascular disease, there is no miRNA-based therapeutics entering clinical trials at present in this area. One of the obstacles impeding development of miRNA-based therapeutics is low delivery efficacy to the vasculature and the likely need for repeated delivery. There are several strategies to deal with miRNA-based therapeutic challenges, which are summarised and discussed in this review [114*]. However, despite the obstacles, pharmacological manipulation of disease-associated miRNAs shows promising therapeutic potential in animal disease models. With more and intensive studies of miRNAs *in vitro* and *in vivo* pre-clinical models and human patients, the application of miRNA-based therapeutics in the clinic can become a reality. For lncRNAs, there are also several obstacles facing the development of lncRNA-based therapeutics. First, the functions and mechanisms used by lncRNAs are much more complex and diversified than miRNAs [115]. In spite of recent studies, lncRNAs are still largely an “unknown” with regard to their cellular functions and molecular mechanisms. Second, the majority of lncRNAs that localise to the nucleus are thought to act as epigenetic regulators [116]. This feature makes it hard to target lncRNAs using siRNA, which is a potential therapeutic strategy. Third, most lncRNAs lack conservation between species, which restricts the utility of pre-clinical disease models. One possible strategy to overcome these issues is to identify the direct targets of disease-associated lncRNAs and use pre-clinical disease models to assess the therapeutic role of these targets in disease pathogenesis and/or use innovative human-based model systems *ex vivo* and *in vitro*.

Conclusions

Recent studies have dramatically improved our understanding of the multifaceted roles that ncRNAs play in pathological vascular remodelling. The expression of several miRNAs have been shown to be dysregulated in various animal disease models as well as in patient samples, while novel direct targets of these disease-associated miRNAs have been validated. Manipulation of these miRNAs in pre-clinical animal models has showed therapeutic benefit. However, there are still some less-understood aspects of miRNA biology that require further study, for example the role(s) of miRNA passenger strands (which in some cases have been shown to be functional), *in vivo* targeting (as most targeted studies of miRNAs are carried out *in vitro*) and miRNA: miRNA or miRNA: lncRNA interactions. Compared with miRNAs, only few LncRNAs have been investigated in depth in vascular disease. The cellular function and biological mechanisms utilised by these lncRNAs still requires further exploration. Taken together, the evidence summarised in this review reveals that targeting ncRNAs may be a promising therapeutic strategy for the treatment of diseases characterised by vascular remodelling.

Key points

- Noncoding RNAs have emerged as critical regulators of diseases characterised by pathological vascular remodelling.
- MiRNAs have been shown to regulate VSMC proliferation, migration, apoptosis and phenotype switching.
- Accumulating studies indicate that lncRNAs may critically regulate vascular pathophysiology. Deep sequencing will identify more lncRNAs involved in the regulation of vascular cell behaviour and disease pathogenesis.
- Exosomal miRNAs from vascular cells play an important role during the development of atherosclerosis and PAH by regulating SMC and EC function.
- Based on extensive studies in pre-clinical models of vascular disease and in patient samples, several clinical trials using various approaches to manipulate ncRNA (e.g. anti-miRNAs, miR mimics) are now in progress.

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Conflicts of interest

None

Table 1 Overview of confirmed target genes of ncRNAs in VSMCs

ncRNA	Target genes	Effect on cell behaviour/neointimal formation
miR-21	PTEN[29, 38**], BCL2[29]	VSMC proliferation and apoptosis
	PDCD4[30, 37**]	VSMC apoptosis, proliferation, and migration
	TPM1[31]	VSMC proliferation and migration
	SP1[32]	VSMC proliferation and differentiation
	DOCK4,5,7[35]	VSMC migration and contractility
	STAT3[36, 37**],PTEN, BMPR2[36]	VSMC proliferation, migration; modulates neointimal formation
	BMPR2, STAB1, YOD1[40]	PASMC proliferation and apoptosis
miR-221	CDKN1B[48]	VSMC proliferation; modulates neointimal formation
miR-30c	PDGFRβ[51]	PASMC proliferation and apoptosis
miR-322	BMPR1α, Smad5[52]	PASMC proliferation and migration

miR-130	CDKN1A[53]	PASMC proliferation
Lnc-*Ang*362	miR-221, mi-222[68]	VSMC proliferation
LincRNA-p21	MDM2[73**]	VSMC proliferation and apoptosis; modulates neointimal formation
SMILR	HAS2[117]	VSMC proliferation

BMPR2 indicates bone morphogenetic protein receptor 2; PTEN, phosphatase and tensin homologue; BCL2, B-cell lymphoma 2; PDCD4, programmed cell death 4; TPM1, tropomyosin 1 (Alpha); SP1, specificity protein 1; DOCK4,5,7, dedicator of cytokinesis 4,5,7; STAT3, signal transducer and activator of transcription 3; STAB1, stabilin-1; YOD1, YOD1 deubiquitinase; CDKN1B, cyclin-dependent kinase inhibitor 1B; PDGFRβ, beta-type platelet-derived growth factor receptor; BMPR1α, bone morphogenetic protein receptor type 1α; CDKN1A, cyclin-dependent kinase inhibitor 1A; MDM2, E3 ubiquitin-protein ligase Mdm2; and HAS2, hyaluronan synthase 2.

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* of special interest
** of outstanding interest

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Figure Legends

Figure 1: Overview of the genome location and functions of lncRNAs

(**A**). Based on their genome locations, lncRNAs can be classified into Sense: transcribed from the sense strand of a protein-coding gene; Antisense: transcribed from the antisense strand of a protein-coding gene; Intronic: transcribed entirely from an intron of a protein-coding gene; Intergenic: transcribed sequences is not located near any other protein-coding loci; Bidirectional: transcribed sequences are located on the opposite strand from a protein-coding gene whose transcription is initiated less than 1000 base pairs away. (**B**). The main functions of lncRNA including SIGNALLING: lncRNA can function as molecular signal or indicator of transcriptional activity; DECOY: lncRNAs can preclude the access of regulatory proteins to DNA; GUIDES: lncRNA are be required for the localisation of specific protein complexes either in *cis* (on neighbouring genes) or in *trans* (on distantly located genes); SCAFFOLDS: lncRNAs can serve as adaptors to bring two or more proteins into discrete complexes.

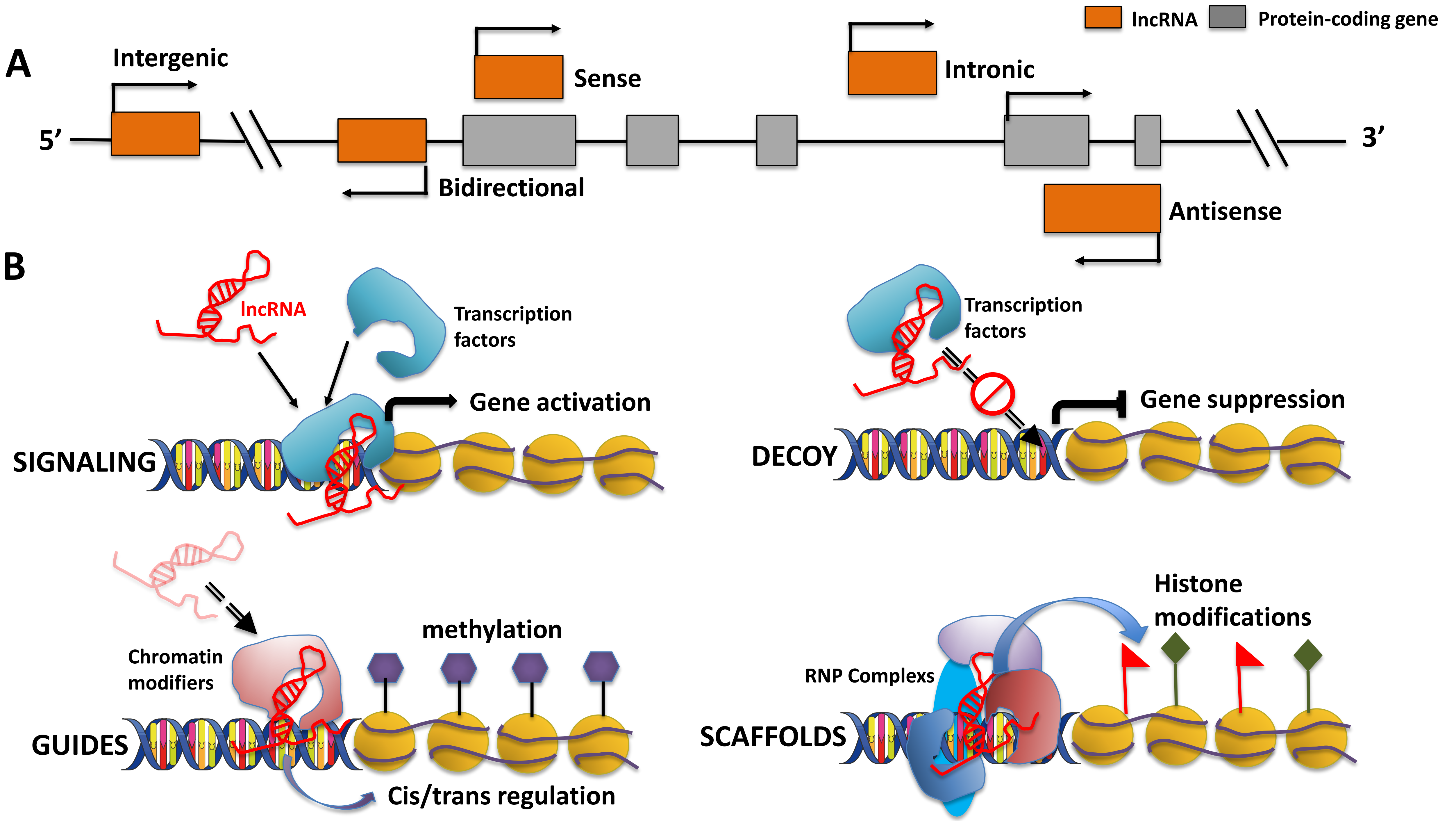
Figure 2: Cell-to-cell communication between vascular smooth muscle and endothelial cells through miRNAs

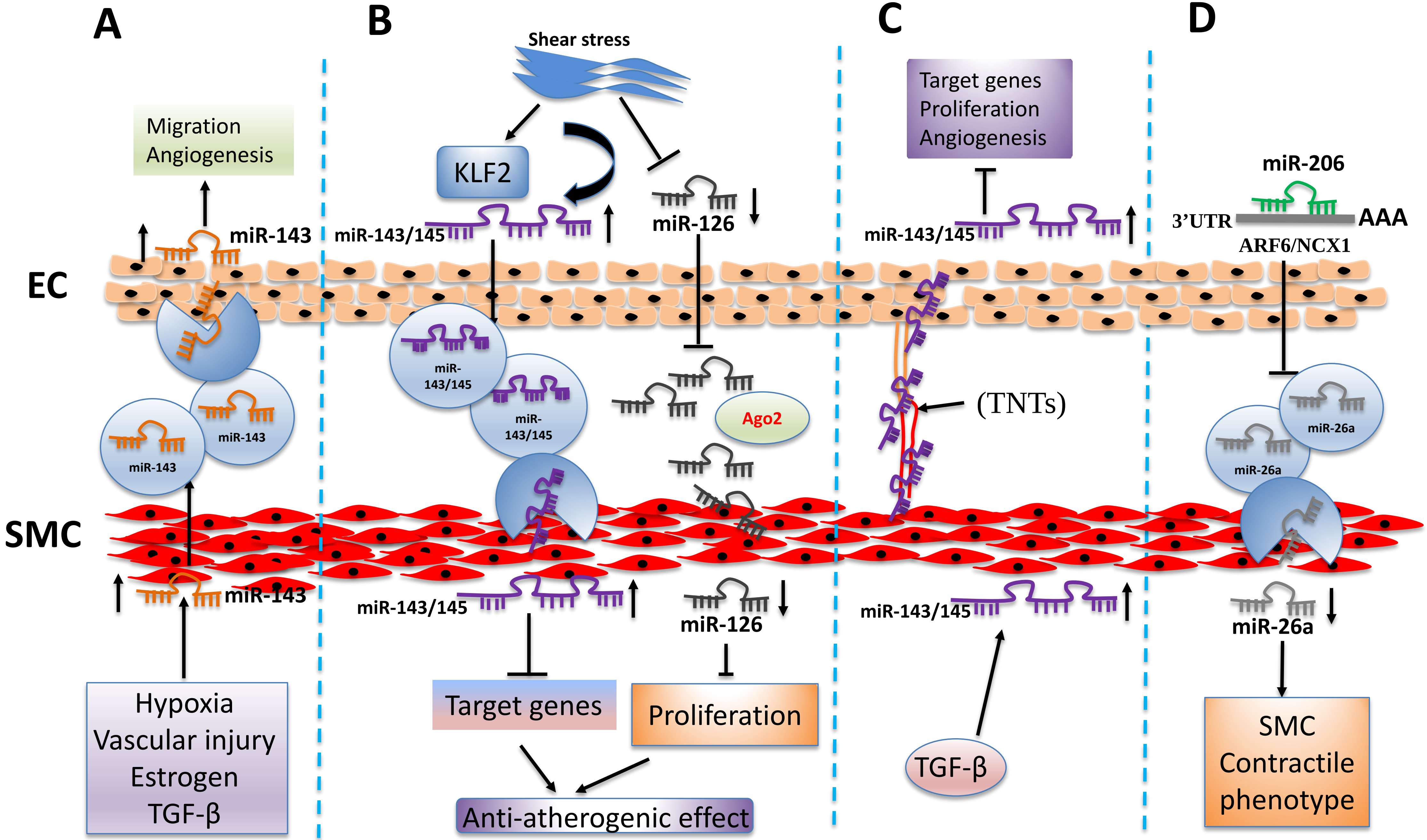
Working models were showed to illustrate cell-to-cell communication between VSMCs and ECs. (**A**). Various stimuli induce exosomal miR-143 secretion in PASMCs, which can be taken up by EC to induce cell migration and angiogenesis [50**]. (**B**). Shear stress can induce KLF2 and miR-143/145 expression and inhibit miR-126 expression in EC. MiR-143/145 and miR-126 transfer to VSMCs through exosomes, and Ago2-facilitated miRNA transmission affects target genes and cellular functions in VSMCs, leading to atheroprotective phenotype [95, 97]. (**C**). TGF-β induced miR-143 expression in VSMCs, which can be transferred to ECs through TNTs to inhibit target gene expression and induce cell proliferation and angiogenesis [96]. (**D**). MiR-206 binding to 3'UTR of ARF6/NCX1 in EC inhibits exosomal miR-26a secretion and uptake into VSMCs, where they regulate the SMC contractile phenotype [98].

Table 1 Overview of confirmed target genes of ncRNAs in VSMCs

ncRNA	Target genes	Effect on cell behaviour/neointimal formation
miR-21	PTEN[29, 38**], BCL2[29]	VSMC proliferation and apoptosis
	PDCD4[30, 37**]	VSMC apoptosis, proliferation, and migration
	TPM1[31]	VSMC proliferation and migration
	SP1[32]	VSMC proliferation and differentiation
	DOCK4,5,7[35]	VSMC migration and contractility
	STAT3[36, 37**],PTEN,	
	BMPR2[36]	VSMC proliferation, migration; modulates neointimal formation
	BMPR2, STAB1, YOD1[40]	PASMC proliferation and apoptosis
miR-221	CDKN1B[48]	VSMC proliferation; modulates neointimal formation
miR-30c	PDGFRβ[51]	PASMC proliferation and apoptosis
miR-322	BMPR1α, Smad5[52]	PASMC proliferation and migration
miR-130	CDKN1A[53]	PASMC proliferation
Lnc-*Ang*362	miR-221, mi-222[68]	VSMC proliferation
LincRNA-p21	MDM2[73**]	VSMC proliferation and apoptosis; modulates neointimal formation
SMILR	HAS2[117]	VSMC proliferation

BMPR2 indicates bone morphogenetic protein receptor 2; PTEN, phosphatase and tensin homologue; BCL2, B-cell lymphoma 2; PDCD4, programmed cell death 4; TPM1, tropomyosin 1 (Alpha); SP1, specificity protein 1; DOCK4,5,7, dedicator of cytokinesis 4,5,7; STAT3, signal transducer and activator of transcription 3; STAB1, stabilin-1; YOD1, YOD1 deubiquitinase; CDKN1B, cyclin-dependent kinase inhibitor 1B; PDGFRβ, beta-type platelet-derived growth factor receptor; BMPR1α, bone morphogenetic protein receptor type 1α; CDKN1A, cyclin-dependent kinase inhibitor 1A; MDM2, E3 ubiquitin-protein ligase Mdm2; and HAS2, hyaluronan synthase 2.


A
lncRNA
Protein-coding gene
Intergenic
Sense
Intronic
5'
3'
Bidirectional
Antisense
B
lncRNA
Transcription factors
Gene activation
SIGNALING
Transcription factors
Gene suppression
DECOY
methylation
Chromatin modifiers
GUIDES
Cis/trans regulation
Histone modifications
RNP Complexs
SCAFFOLDS


A
B
C
D
Shear stress
Migration
Angiogenesis
KLF2
Target genes
Proliferation
Angiogenesis
miR-206
3'UTR
AAA
ARF6/NCX1
miR-143
miR-143/145
miR-126
miR-143/145
EC
miR-143
miR-143
miR-143/145
miR-143/145
Ago2
(TNTs)
miR-26a
miR-26a
SMC
miR-143
miR-143/145
miR-126
miR-143/145
miR-26a
Hypoxia
Vascular injury
Estrogen
TGF-β
Target genes
Proliferation
Anti-atherogenic effect
TGF-β
SMC
Contractile
phenotype